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# Bitonic dose–response functions for reinforcing and self-reported effects of nitrous oxide in humans

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**Abstract**

The reinforcing and self-reported effects of nitrous oxide (10%, 30%, and 50% $N_2O$ in $O_2$) were examined in 13 humans. During each of three sessions, subjects sampled one dose of $N_2O$ and 100% $O_2$ (placebo) for 10 min each, separated by 30-min recovery periods. The agents were identified by letter code, and later in the session, subjects chose nine times, once every 5 min, among $N_2O$ (e.g., "Agent A"), placebo (e.g., "Agent B"), or "neither" (also 100% $O_2$, identified as "drug-free air"). Self-reported and psychomotor effects were measured at various times. Dose–response functions varied across subjects and included bitonic, monotonic increasing, monotonic decreasing, U-shaped, and flat dose–response functions for reinforcing and/or self-reported effects. For subjects who showed bitonic reinforcing effects, the descending limb of the dose–response function could not be attributed to behavioral impairment. This study replicates previous studies showing dose-dependent effects of $N_2O$, as well as between-subject variability in $N_2O$ effects. Bitonic dose–response functions for some subjects extend the generality of the phenomenon of bitonicity of drug effects to $N_2O$ effects in humans.


*Keywords:* Drug self-administration; Dose–response curve; Bitonic; Inverted U; Nitrous oxide; Reinforcing effects; Self-reported effects; Choice; Humans

## 1. Introduction

Frequently, behavioral effects of drugs, including reinforcing effects, are bitonic; that is, low to moderate doses increase behavioral effects, and higher doses decrease effects, resulting in an inverted U-shaped dose–response function (Branch, 1991; Carlton, 1983; Meisch, 1987). There are numerous examples of bitonic reinforcing effects of drugs in nonhumans, such as cocaine and heroin in rhesus monkeys (Negus et al., 1995), ethanol in rats (Brown et al., 1998), nicotine in rats (Watkins et al., 1999), and $N_2O$ in rhesus monkeys (Grubman and Woods, 1981). (For other recent examples, see Birmingham et al., 1998; Briscoe et al., 1998; Pogorelov and Kovalev, 1999; Rose and Corrigall, 1997; Shoaib et al., 1997). In the study with $N_2O$ and rhesus monkeys, fixed-ratio (FR) responding was measured when varying concentrations of $N_2O$ were the programmed consequences. Nominal concentrations (the actual amount of $N_2O$ pumped into the monkey's helmet, mixed with $O_2$) ranged from 0% to 100% $N_2O$ and resulted in maximum concentrations in the helmet after $\geq$2 min of 0–59% $N_2O$. The authors found inverted U-shaped dose–response functions for the two subjects whose behavior was maintained by an FR 1 schedule of $N_2O$ delivery (peak response rates at nominal concentrations of 8–33% $N_2O$). For the two subjects whose responding was maintained by FR 30, response rates peaked between 33% and 67% $N_2O$ and decreased slightly at the 100% $N_2O$ concentration (Grubman and Woods, 1981). When humans are the subjects in drug self-administration studies, though, monotonic, increasing reinforcing effects seem to be more common than bitonic reinforcing effects (cf., Comer et al., 1997; Griffiths et al., 1979; Hart et al., 2000; Heishman et al., 2000; McLeod and Griffiths, 1983), probably due to constraints on the size of the dose that can be safely and ethically administered to humans (Henningfield et al., 1991).

We recently conducted a study that examined the reinforcing and self-reported effects of different doses of $N_2O$ (0–40% $N_2O$ in $O_2$) in nondrug-abusing humans (Walker and Zacny, 2002). That study showed dose-related increases in choice of $N_2O$ for all subjects combined (monotonic, increasing dose–response function), as well as individual differ-

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ences in the extent to which, and the dose(s) at which, $N_2O$ functioned as a reinforcer. Only 1 of 20 subjects produced an inverted U-shaped dose–response function for choice; for the other 15 subjects who showed evidence of $N_2O$'s reinforcing effects, choice did not decrease at the largest dose(s). The present study was conducted to see if the dose–response function for choice in this subject population would show bitonicity if we tested a higher dose than was used in the previous study. We tested 10%, 30%, and 50% $N_2O$, compared with placebo (i.e., 100% $O_2$), using a methodology identical to the previous study. Because the descending limb of the dose–response curve for reinforcing effects of drugs is frequently attributed to the behavior-disrupting effects of the drugs (Griffiths et al., 1979; Henningfield et al., 1991), we assessed psychomotor performance as an objective measure of behavioral impairment. In addition, the behavioral requirement for choice in the present study was circling the chosen option on a paper-and-pencil form, rather than a complex or effortful response or a sequence of responses that could be disrupted by the direct effects of the drug. If subjects' ability to make the choice was not disrupted, then the descending limb of the dose–response function could not be attributed to the direct behavior-impairing effects of $N_2O$.

Another purpose of the present study was to assess whether the self-reported effects of $N_2O$ would also show a bitonic dose–response function if we tested a higher dose than was tested previously. Other effects of $N_2O$ than its reinforcing effects have been shown to be bitonic in nonhumans, such as locomotor effects in mice (Czech and Green, 1992; Czech and Quock, 1993); therefore, we predicted that some subjects would show bitonic dose–response functions for self-reported effects, as well.

## 2. Methods

### 2.1. Subjects

The study was approved by the local Institutional Review Board and was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. Seven female and six male healthy volunteers, 21–34 (mean = 26) years old, completed the study. Self-reported current alcohol usage ranged from 1 to 12 (mean = 4) drinks per week, five subjects reported smoking five or fewer tobacco cigarettes per day, and five subjects reported smoking less than five marijuana cigarettes per week. Self-reported lifetime recreational drug usage indicated no history of drug addiction or other drug-related problems. Seven subjects reported having received $N_2O$ during medical/dental procedures.

### 2.2. Apparatus/setting

The experimental room contained a reclining chair, a television, an anesthesia machine, and resuscitative equipment. The anesthesia machine was located behind the recliner, and anything on the machine that might identify the gases being delivered was covered by a towel so that the subjects and technician were blind to the agents being administered. The anesthetist delivering the inhaled agents sat behind the subject next to the anesthesia machine. The research technician sat at a desk about 1 m from the recliner and monitored subjects continuously.

### 2.3. Design

A randomized, placebo-controlled, crossover design was used. Three $N_2O$ doses were examined, in comparison with placebo, across three different sessions. The doses used in the present study were 10%, 30%, and 50% $N_2O$ in $O_2$. One of the three doses was sampled during each session, and the vehicle (100% $O_2$, placebo) was also sampled. We used 100% $O_2$, rather than air, as our placebo condition because $O_2$ is the vehicle and because we have found no differences in mood or psychomotor performance between 100% $O_2$ and compressed air (Dohrn et al., 1992). Gases were administered under normal atmospheric pressure. Each sample was 10 min long, and later in the session, subjects could choose on nine consecutive 5-min choice trials what they wanted to inhale (one of the two sampled agents or neither, in which case they received 100% $O_2$). A Latin square was used to randomize the order of dose conditions, and the order of $N_2O$ vs. placebo samples was counterbalanced across sessions. Placebo and the active drug were identified as "Agents A and B" during the first session, "Agents C and D" during the second session, and "Agents E and F" during the third session. Subjects were told that "the agents may or may not contain a drug." Subjects also had the option of neither and were told that if they chose neither, they would "receive air that has no drug in it." Subjects were told that "the agents, which may or may not contain a drug, may be the same or different during different sessions."

The rationale for the particular choice procedure used in the present study (i.e., drug vs. placebo vs. neither) is as follows. Traditional discrete-trial choice procedures require subjects to choose between drug and placebo, and reinforcing effects are inferred if subjects choose drug more than placebo. Such "forced-choice" procedures require subjects to choose placebo on trials in which they do not choose drug, making choice behavior difficult to interpret (Spiga and Roache, 1997). In the present study, the option of "neither" eliminated the forced consumption of placebo, allowing the placebo to function as a negative control (i.e., little or no choice of placebo was possible, even if subjects never or rarely chose drug). In this way, the number of drug choices, relative to the number of placebo choices, is a more meaningful indicator of a drug's reinforcing effects than if placebo choice were directly dependent on drug choice, as in traditional, "forced-choice" procedures (Spiga and Roache, 1997; Walker and Zacny, 2001a,b).

### 2.4. Sessions

The study consisted of three 4-h sessions, separated by at least 3 days. Subjects arrived at the laboratory at 1300 h and delivered breath and urine samples (for pregnancy and drug screening) and signed a compliance form stating that they had complied with our presession fasting requirements (no eating for 4 h, no drinking for 2 h, no drugs for 24 h). Subjects sat in the recliner and were fitted with a blood pressure cuff, pulse oximeter, and the anesthesia mask, and a baseline period of testing began. The anesthetist delivered 100% $O_2$ through the mask, and subjects were told by the research technician, "You are now breathing drug-free air." Dependent measures were assessed during this baseline period, which lasted approximately 5 min.

Immediately following the baseline period, a sampling period began. Subjects were told by the research technician, "For the next 10 min you will be inhaling Agent __ (e.g., A), which may or may not contain a drug," and the anesthetist began administering the first agent ($N_2O$ or placebo). Five minutes into the sampling period, dependent measures were assessed. After 10 min, the mask was removed, and a 30-min recovery period began, during which dependent measures were assessed periodically. After the 30-min recovery period, the mask was replaced, and another baseline period occurred, which was identical to the one described above. The second sampling period followed, which was identical to the first except for the agent being sampled [placebo or $N_2O$ (e.g., Agent B)]. Another 30-min recovery period (mask off) followed. Then the mask was replaced, and the choice period began. At the start of the choice period, subjects were told "The inhalation period will be 45 min long. Every 5 min, I will ask you if you would like to inhale Agent __ (e.g., A), Agent __ (e.g., B), or neither for the next 5 min. I will ask you this every 5 min until the 45 min is up. Please circle your choice on this form." The research technician then asked the subject which agent he/she would like to inhale, the subject circled his/her choice on the form, the anesthetist administered the appropriate agent, and the technician informed the subject, "You are now inhaling Agent __ (or drug-free air) for the next 5 min." Nine such trials occurred during the 45-min choice period, and a 60-min recovery period followed. Subjects were transported home by a livery service after the anesthetist approved their dismissal from the laboratory.

### 2.5. Dependent measures

Dependent measures included choice, self-reported effects, psychomotor performance, and physiological measures. Choice consisted of the total number of choices of $N_2O$, placebo, and neither during each session.

Self-reported effects were measured by three instruments. One, the Visual Analog Scale (VAS) questionnaire, consisted of twenty-four 100-mm lines, each labeled with a drug effect, symptom, or feeling. Subjects were instructed to place a mark on each line indicating how they felt at the moment, ranging from 0 mm (*not at all*) to 100 mm (*extremely*). VAS items were stimulated, high, heavy/sluggish, sedated, lightheaded, tingling, confused, drunk, elated, nauseous, dreamy, coasting, floating, sleepy, down, having pleasant thoughts, having unpleasant thoughts, having pleasant bodily sensations, having unpleasant bodily sensations, feel good, feel bad, difficulty concentrating, in control of thoughts, and in control of body. Two, the Drug Effect/Drug Liking/Inhale Again (DEL) questionnaire assessed the extent to which subjects currently felt a drug effect on a scale of 1 (*I feel no effect from it at all*) to 5 (*I feel a very strong effect*). Subjects were also asked to place a mark on a 100-mm line indicating how much they liked the drug effect, ranging from 0 mm (*dislike a lot*) through 50 mm (*neutral*) to 100 mm (*like a lot*), and how much they "would want to inhale the agent again on another session, if given the opportunity," ranging from 0 mm (*definitely would not*) through 50 mm (*do not care*) to 100 mm (*definitely would*). These two self-report measures were assessed at baseline; 5 min into the sampling period; 5 and 20 min after each sampling period; and 5, 30, and 60 min after the choice period; however, data presented are from sampling periods only. The VAS and DEL questionnaires took approximately 90 and 30 s, respectively, to complete.

The third self-report instrument was the Inhalant Drug Effects questionnaire (Block et al., 1990), which is sensitive to the psychedelic and somatic effects of $N_2O$ (Atkinson et al., 1977). The questionnaire consists of 53 brief descriptions of possible drug effects, and subjects were asked to place a checkmark next to those items they had experienced since the onset of the inhalation period. The descriptions fall into seven categories: body awareness/image; time perception; dreamy, detached reverie; cognitive–motor deficiency; happy, euphoric mood; sensation/perception; and adverse, dysphoric effects. The total number of check marks in each category was summed to yield seven different scores. This test was administered 8 min after the end of each sampling period and took about 90–120 s to complete.

The psychomotor test was the Digit Symbol Substitution Test (DSST), a 60-s paper-and-pencil test that required subjects to replace digits with corresponding symbols according to a digit symbol code listed on the top of the paper (Wechsler, 1958). The DSST was administered immediately after the VAS and DEL questionnaires.

Physiological measures included blood pressure, heart rate, and arterial $O_2$ saturation. These measures were taken immediately before the VAS and DEL questionnaires were administered.

### 2.6. Data analysis

Data were analyzed by visual inspection of dose–response functions for individual subjects and for the group. Choice data were analyzed statistically by repeated-measures analysis of variance (ANOVA) with the factors, $N_2O$ Condition (10%, 30%, 50%) and Agent ($N_2O$, placebo, neither).

Self-reported effects, DSST performance, and physiological measures during sampling periods were analyzed by repeated-measures ANOVA with the factors, $N_2O$ Condition and Sample ($N_2O$, placebo). When results of ANOVAs were statistically significant ($P \leq .05$), Tukey post-hoc tests identified which differences were statistically significant.

## 3. Results

### 3.1. Choice

Twelve subjects completed all choice periods, and one subject (Subject 2) ended the choice period during the last session after choosing 50% $N_2O$ three times then placebo once. Fig. 1 shows dose–response functions for $N_2O$ choice for individual subjects. Six subjects (Subjects 1, 2, 4, 5, 7, and 8) showed evidence of bitonic reinforcing effects of $N_2O$, that is, increased $N_2O$ choice relative to placebo following the small to medium dose(s), followed by a decreasing trend at the medium to large dose(s). Three subjects (Subjects 3, 6, and 12) showed monotonic increases in $N_2O$ choice as a function of dose [although Subject 12 showed only a very small increase (from 0 to 1 choice) at the largest dose]. Subject 14 showed a somewhat U-shaped dose–response curve, with increased $N_2O$ choice relative to placebo at the two highest doses, with a small decrease relative to placebo at 10% $N_2O$. Three subjects (Subjects 9, 10, and 13) showed no evidence of $N_2O$'s reinforcing effects (no dose of $N_2O$ was chosen more than placebo), and these subjects chose "neither" on 20, 27, and 22 (out of 27) choice trials, respectively.

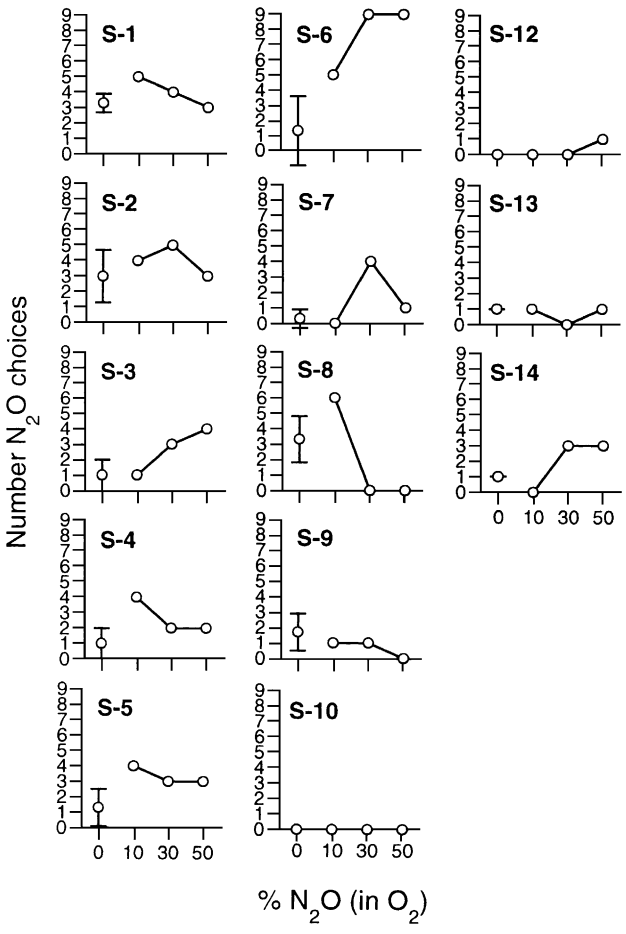


Fig. 1. Dose–response functions for $N_2O$ choice for individual subjects. Points above 0% represent the mean number of times placebo was chosen across the three sessions. Bars around those points represent standard deviations. Points above the other doses (10–50%) represent the total number of times those doses of $N_2O$ were chosen.

When data from all subjects were combined, $N_2O$ was chosen more than placebo, and "neither" was chosen more than $N_2O$ or placebo, regardless of the concentration being tested (Agent: $P < .05$). Overall, $N_2O$ was chosen on 27% of choice trials, placebo was chosen on 16% of choice trials, and "neither" was chosen on 57% of choice trials.

### 3.2. Self-reported drug effects

$N_2O$ produced dose-related effects typical of those observed in our previous $N_2O$ studies (e.g., Walker and Zacny, 2002). Specifically, mean ratings of the following items increased during the $N_2O$ sample, but not during the placebo sample, as a function of dose (i.e., statistically significant $N_2O$ Condition × Sample interaction): the VAS items, stimulated, high, tingling, confused, elated, coasting, floating, and difficulty concentrating (all $P$s $< .05$); the Likert scale item, drug effect strength ($P < .001$); and all scales of the Inhalant Drug Effects questionnaire (all $P$s $< .05$). Mean VAS ratings of heavy/sluggish, lightheaded, drunk, dreamy, and having pleasant bodily sensations also showed this pattern, but the interaction effects only approached statistical significance ($P \leq .10$). Mean ratings of "in control of thoughts" and "in control of body" decreased as a function of dose during the $N_2O$, but not placebo, sample ($N_2O$ Condition × Sample interaction: $P \leq .05$). Although there was some variability in the shape of the dose–response functions across subjects, overall means/trends were representative of data for the majority of individual subjects.

Three items showed evidence of bitonic effects when both group and individual subject data were examined: VAS ratings of having pleasant thoughts and feel good, and drug liking. Fig. 2 shows ratings of having pleasant thoughts and feel good, as a function of dose for individual subjects. For ratings of having pleasant thoughts, five subjects showed bitonicity in the dose–response functions (Subjects 4, 5, 7, 8, and 12), and one subject (Subject 13) showed a trend toward bitonicity. Three subjects (Subjects 2, 9, and 10) showed no change in ratings of having pleasant thoughts at the smaller doses and decreased ratings during the 50% $N_2O$ sample. Two subjects (Subjects 6 and 14) showed monotonic increases, and two subjects (Subjects 1 and 3) showed no change in ratings of having pleasant thoughts across doses. For ratings of feel good, two subjects (Subjects 8 and 12) showed inverted U-shaped dose–response functions, and three subjects (Subjects 4, 9, and 13) showed a trend toward

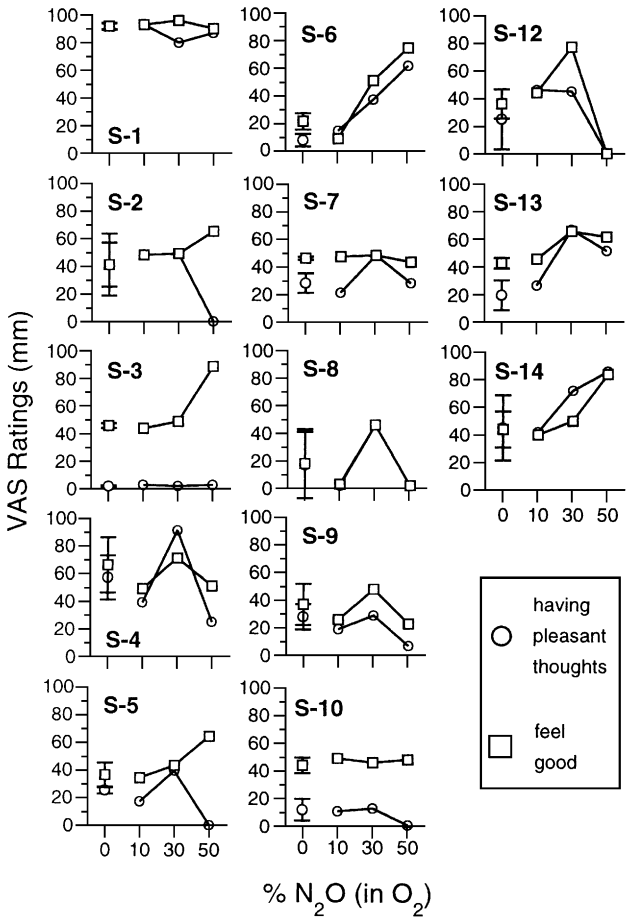


Fig. 2. VAS ratings of having pleasant thoughts and feel good as a function of dose for individual subjects. Points above 0% represent the mean rating during the placebo sample across the three sessions. Bars around those points represent standard deviations. Points above the other doses (10–50%) represent the rating when those doses of $N_2O$ were sampled.

an inverted U. Five subjects (Subjects 2, 3, 5, 6, and 14) showed monotonic increases, and three subjects (Subjects 1, 7, 10) showed no change in ratings of feel good across doses. When data from all subjects were combined, mean ratings of these VAS items increased relative to placebo from 10% to 30% $N_2O$, then decreased during the 50% $N_2O$ condition (having pleasant thoughts, $N_2O$ Condition: $P<.01$; feel good, $N_2O$ Condition: $P<.05$, Sample: $P<.05$).

For ratings of drug liking (data not shown), visual inspection of data for individual subjects showed that four subjects (Subjects 7, 8, 10, and 12) showed clear inverted U-shaped dose–response functions for drug liking during the sample, and one subject (Subject 1) showed decreased ratings during the 50% sample with no increases at the smaller doses. Seven subjects (Subjects 2, 4, 5, 6, 9, 13, and 14) showed monotonic increases in ratings across doses, and one subject (Subject 3) rated approximately 50 mm (neutral) for all $N_2O$ doses. As with mean ratings of pleasant thoughts and feel good, mean ratings of drug liking tended to increase from 10% to 30% $N_2O$ then to decrease during the 50% $N_2O$ condition ($N_2O$ Condition: $P<.10$, Sample: $P=.01$). Individual subject ratings of wanting to inhale the agent again tracked closely ratings of drug liking; however, no statistically significant effects were observed for that dependent measure.

### 3.3. Psychomotor and physiological effects

The number of symbols drawn correctly on the DSST decreased as a function of dose during the $N_2O$, but not placebo, sample ($N_2O$ Condition × Sample: $P<.001$). The mean number of symbols drawn correctly was 55 during the placebo samples and 53, 49, and 33 during the 10%, 30%, and 50% $N_2O$ samples, respectively. All subjects showed this monotonic decreasing dose–response function. Physiological effects of $N_2O$ were not clinically significant: although statistically significant increases were observed for diastolic blood pressure and pulse ($N_2O$ Condition: both $P$s $<.01$; Sample: both $P$s $<.01$), the largest difference from placebo was less than 15% of placebo values.

## 4. Discussion

Reinforcing and self-reported effects of $N_2O$ were dose-dependent and varied across subjects. We saw bitonic, as well as monotonic increasing, monotonic decreasing, U-shaped, and "flat" (no effects) dose–response functions for choice and/or self-reported effects. The present results replicate those of previous studies that showed dose-dependent effects of $N_2O$, as well as between-subject variability in reinforcing and self-reported effects of $N_2O$ (Dohrn et al., 1993a,b; Walker and Zacny, 2001b, 2002; Zacny et al., 1996) and other drugs (Chait, 1993; de Wit et al., 1986a,b, 1987, 1989a,b; Griffiths and Woodson, 1988a) in nondrug-abusing volunteers. The present data also replicate previous studies that showed bitonic, as well as monotonic increasing, dose–response functions for reinforcing and other behavioral effects of $N_2O$ in nonhumans (Czech and Green, 1992; Czech and Quock, 1993; Grubman and Woods, 1981; Quock et al., 1987, 1992; Wood et al., 1977). The fact that some subjects showed bitonic dose–response functions for reinforcing and/or self-reported effects of $N_2O$ extends the generality of the phenomenon of bitonicity of behavioral effects of drugs to $N_2O$ effects in humans.

Bitonic dose–response functions for reinforcing effects of drugs have been observed in humans with oral caffeine and intravenous nicotine (Goldberg and Henningfield, 1988; Griffiths and Woodson, 1988b; Griffiths et al., 1986; Henningfield et al., 1983; Rose and Corrigall, 1997). Fischman and Foltin (1992), however, failed to observe a descending limb on the dose–response function for cocaine self-administration by cocaine abusers, probably because increases in heart rate and blood pressure limited the cumulative dose that could be administered. In the present study, 50% $N_2O$ had little effect on vital signs and allowed bitonic reinforcing effects to be observed in some subjects with no risk to

subject safety. In addition, there was an absence of profound behavioral impairment that could account for the decrease in reinforcing effects at the largest dose. Nitrous oxide did produce substantial psychomotor impairment as measured by the DSST; however, this impairment did not affect subjects' ability to meet the response requirement for choice, a result that is significant because the descending limb of the bitonic dose–response function is frequently attributed to the behavior-disrupting effects of the drug (Branch, 1991; Griffiths et al., 1979; Henningfield et al., 1991). Such data are consistent with the possibility discussed by Katz (1990) and Stafford et al. (1998) that the descending limb of the inverted U may reflect the decreasing effectiveness of the larger drug doses as reinforcers, rather than necessarily being a result of the direct behavior-impairing effects of the drug.

One reason that larger doses may be less effective reinforcers than smaller doses may be that the larger doses produce aversive effects or satiation that is absent following administration of the smaller doses (Griffiths and Woodson, 1988a; Griffiths et al., 1979; Henningfield et al., 1983, 1991; Katz, 1990; Rose and Corrigall, 1997; Stafford et al., 1998). The most obvious suggestion of aversive effects in the present study was removal of the mask by three subjects during inhalation of 50% $N_2O$ (Subject 2 and two other subjects who dropped out of the study because of unpleasant effects of that dose). Mask removal was accompanied by verbal reports that supported the possibility of aversive effects, such as reports of disliking the drug (Subject 2), feeling "like I would pass out," and feeling "scared." Although verbal reports of drug effects cannot provide direct evidence of reinforcing or aversive effects, these verbal reports, in combination with mask removal, support the hypothesis that 50% $N_2O$ produced aversive effects in some subjects. The possibility of satiation was also apparent. For most of the subjects who chose $N_2O$, those choices were interspersed with choices of neither or placebo, and verbal reports during debriefing interviews indicated that some subjects were "taking a break" from the drug. Similar results (choices of $N_2O$ separated by choices of a no-drug alternative, along with reports of wanting a break from the drug) have been reported previously in this laboratory with smaller doses of $N_2O$ (Walker and Zacny, 2001a).

Not all subjects showed bitonicity of drug effects, and for the subjects who did, sometimes the dose–response functions were not clear inverted Us but, rather, showed "trends" in the direction of bitonicity. If we had tested a higher dose, we are certain that more subjects would have produced inverted U-shaped dose–response curves; however, doses higher than 50% $N_2O$ can be anesthetic in some subjects (Parbrook, 1967), and we did not want to confound dose with unconsciousness in this study. Another potential methodological improvement would have been to replicate the dose conditions within-subject. However, a previous study conducted in this laboratory using methodology virtually identical to the present one found that choice of 30% $N_2O$ was remarkably consistent within-subject, and self-reported effects of that dose varied quantitatively but not qualitatively, across five separate sessions (Walker and Zacny, 2001b). That study suggests strongly, therefore, that the present results are representative of data during subsequent replications.

A final caveat to the present study is the lack of a placebo control session. Although reinforcing and subjective effects of placebo were tested in each session, they were always tested in the context of a session in which an active drug dose was also administered. This does not appear to be a problem, given that placebo choice was similar across sessions within the same subjects, suggesting that placebo choice did not depend on the other dose being sampled in that session. In addition, the order of drug conditions was randomized across subjects, and the order of drug and placebo samples was counterbalanced across sessions within-subject; therefore, we would not expect the other sample agent to affect placebo choice in a systematic fashion.

In summary, although the bitonicity of $N_2O$ effects in the present study was not as robust as it might have been under different experimental conditions, some subjects did show bitonic drug effects, and some subjects showed trends toward bitonicity. These results indicate that it is possible to find inverted U-shaped dose–response functions for reinforcing and self-reported effects of drugs in humans at doses that are safe to administer. The fact that subjects were capable of making the choice response shows that an argument based on the direct behavior-impairing effects of the drug cannot explain the decline in reinforcing effectiveness of the higher dose(s). The present study provides further evidence of the generality of the phenomenon of bitonicity of behavioral effects of drugs and illustrates the similarity of results of behavioral experiments with human and nonhuman subjects.

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